VIABILITY OF MICROORGANISMS IN THE DESERT SOILS OF TURKMENIA

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ABSTRACT. The viability of microorganisms under conditions of extreme dryness, irradiation and temperature, approximating those on Mars, was investigated in the Darvaza, Repetek and Dushak regions of Turkmenia, USSR in July, 1964. Air and soil temperature, CO_2 liberated by the soil, and total N_2 content were measured. It is found that water is more important for mass reproduction of microorganisms than organic matter.

In analyzing the possibility that terrestrial forms of microorganisms may /503* exist under conditions approximating the climate of Mars, we encountered the problem of whether bacterial cells can multiply under conditions of minimum moisture. It was of interest to determine at what level of natural moisture the vital activity of microorganisms is possible on earth. For this purpose, an expedition was undertaken into the central regions of the desert zone of Turkmenia. The literature is lacking in data on microbiological investigations in the sands of the deserts of the Soviet Union. There is the great work by Feher (Ref. 6) on microbiological activity in the sands of the Sahara Desert. The matter of the moisture limits at which microorganism growth is possible has remained moot since the author was unsuccessful in carrying out the proper experiments.

The aim of our work has been to describe the critical conditions on earth at which the existence of microorganisms is possible, and to determine the nature of microbiological activity under climatic conditions which are extreme in the sense of moisture, temperature, and irradiation. An important factor is the temperature differential, which is most clearly pronounced under the continental climatic conditions of the deserts. There is, of course, no place on earth where the daily temperature differential is 100°C, as it is on Mars, but a certain approximation appears realistic.

Method

The following work was performed on the expedition:

1. Determination of soil humidity;
2. Determination of soil and air temperature;
3. Selection of soil specimens to estimate the number of microorganisms;

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* Numbers in the margin indicate pagination in the original foreign text.

4. Determination of soil "respiration" (liberation of CO_2); and

5. Determination of the potentialities of microorganism multiplication on adding organic matter and water to the soil specimens.

The investigations were conducted at three sites in Turkmenia: the settlement of Darvaza, a region of moving sands 200 km from Ashkhabad; the station of Repetek, territory of the desert reservation of the Academy of Sciences of the Turkmen SSR, where the sands are fixed and mobile only at spots; the region of Mt. Dushak, at a height of 2100 meters in the Kopet-Dagh chain, where the soil is a light, brown loam with an admixture of marl. The expedition worked from July 5 to 14, 1964. This period was not arbitrarily chosen. July is the month of highest temperatures on Turkmenian territory and of the maximum total insolation dosage (Ref. 1). Although intensity of insolation is at a maximum in the winter months (Ref. 4) because of the greater dustiness of the atmosphere over the deserts in summer, the total irradiation dosage during a day is at a maximum in June-July: 764-805 cal/sq. cm (Ref. 1). The summer months are simultaneously the hottest and the driest -- e.g., atmospheric humidity in Darvaza in summer is about 15%, but in Repetek it goes down to 3%

The hygroscopic coefficient of the sands was determined by reducing specimens to a constant weight in a drying oven at 105°C. Humidity was expressed in percentages of absolute dry weight. /504

Temperature of the air and of the upper layers of the soil was determined before starting to select samplings at the time of maximum insolation (2:00 p.m., local time), at sundown (8:00 p.m.), and also at night.

Samplings for determining the total number of microorganisms were taken from the surface, and toluene was added so that the cell count in the specimens would not change during transportation.

The CO_2 liberated by the soils was measured by Shtatnov's method (Ref. 5) based on the absorption by an alkali solution of the carbon dioxide liberated in a closed volume. The tests were made at the experimental sites directly under field conditions, and later with the same soils in the laboratory. The experiments on the liberated CO_2 were made during the night hours. The chief reason for this was the need to eliminate the severe heating in daytime from the closed space in which CO_2 absorption was proceeding. To be sure, at night CO_2 may also be liberated by the respiration of algae whose presence in the sands is probable (Ref. 6), but when analyzing the microbiological preparations we detected no algae in the specimens investigated. Therefore, our findings characterize the activity of other microorganisms. The experiments to determine "respiration" of the deserts lasted from 6 to 16 hours.

The estimate of the specimens' organic substance content was made indirectly from the total amount of nitrogen determined by the standard Kjeldahl

method.

The last observational variant was planned as follows. The preparations for the expedition consisted of preparing test tubes containing different soil specimens weighing 5 grams each and having a given humidity -- 0, 1, 5, and 10%. The soils for these moisture standards were so chosen that the amount of organic material in them varied drastically. Soil from a mountain wilderness, lava, limonite, and ordinary garden soil were used. One-gram amounts of the soil specimens to be tested were introduced into test tubes with a previously determined quantity of organic material of natural origin and definite humidity. Analysis of the content of the test tubes after a certain storage period would reveal the importance of such factors as organic matter and moisture for the development of microorganisms. Limonite and lava soil, poor in organic material, were used in order to obtain an idea of the possibility of terrestrial microorganisms' reproducing in soils whose composition is similar to those assumed to exist on Mars.

Results

To understand the nature of the climatic conditions during selection of the samplings for microbiological analysis, we have presented data on the temperatures of the soil and air at the sites where the work was conducted (Table 1).

TABLE 1. TEMPERATURES OF SOIL AND AIR DURING INVESTIGATIONS

Site	Nature of Ground	Soil Temp. C°			Air Temp. C°		
		at 2:00 p.m.	at 8:00 p.m.	at night	at 2:00 p.m.	at 8:00 p.m.	at night
Darvaza	Moving sands without plant life.	44	37	31	38	31	29
Repetek	Fixed sands; plant life exists.	60	37	27	39	36.5	20
Dushak	Stony gray loam; juniper bushes.	49	22	16	25	19	19

It is evident from Table 1 that the maximum temperature differential in the transition from day to night was observed in Repetek and Mt. Dushak soils, and was 33°C. Air temperature during the twenty-four hours varied less drastically, and the jump did not exceed 19°C. The abrupt temperature change in the topmost soil layers, which were the subject of the investigation, is the reason for their moisture variations (Table 2).

The quantity of microorganism cells, determined by the direct counting method, does not always follow the variation in soil moisture (Table 3), but /505

a general tendency is noticeable: the cell count is higher in soil with an elevated moisture content.

The presence of microorganism cells in the tested soils which seem quite normal when fixed and stained still does not give a sufficiently complete idea of the microbiological processes which occur in the desert. It is easy to imagine that these cells are in a state of anabiosis as a result of the minimum moisture. Their viability is demonstrated by the presence of colony growth on the surface of agar medium in dishes. The growth conditions in dishes of meat-peptone agar, of course, represent an extreme deviation in terms of nutriment, temperature, and humidity from the conditions of the natural habitats of the microorganisms investigated. A rise in incubation temperature to 37°C did not result in an increase in the number of colonies in the dishes; on the contrary, their number became smaller. If, moreover, at 28°C fungi and actinomycetes appeared in addition to the colonies of bacteria, at 37°C, on the other hand, not a single fungus colony was recorded.

TABLE 2. MOISTURE OF TOP SOIL LAYER AT DIFFERENT TIMES OF THE DAY

Site	Humidity, %	
	at 2:00 p.m.	at 8:00 p.m.
Darvaza	0.188-0.196	0.221-0.230
Repetek	0.096-0.125	0.147-0.184
Dushak	1.073-1.785	1.751-2.688

TABLE 3. TOTAL NUMBER OF CELLS AT DIFFERENT HOURS OF THE DAY IN MILLIONS PER GRAM OF SOIL

Site	Number of Cells, %	
	at 2:00 p.m.	at 8:00 p.m.
Darvaza	63.5	154.5
Repetek	84.1	28.4
Dushak	187.8	526.7

The variety of colonies cultered on the meat-peptone agar surface was extremely insignificant. Only about 30 morphologically distinct strains were successfully isolated. As a rule these were flat, slimy colonies which were weakly colored in pinkish or yellowish tones. They appeared on the second day after inoculation. The number of colonies in the dishes, even after inoculations of the first culture, did not exceed ten. The limited number of

TABLE 4. INTENSITY OF CO_2 LIBERATION AND TOTAL NITROGEN CONTENT IN DESERT SOILS

Site	Mg of CO_2 liberated per/m^2 hour	% of total nitrogen
Darvaza	37.8	0.0337
Repetek	34.6	0.0476
Dushak	74.5	1.0621

colonies cultured provides no basis for assuming that the results of the conversion per gram of soil are reliable. To obtain a conception of the viability potentials of microorganisms inhabiting the desert sands, it is better to deal with the results of experiments to determine the CO_2 liberated from the soil during the night hours (Table 4).

The data of Table 4 indicate that the microbiological process of carbon dioxide liberation in desert sands is distinguished by rather perceptible intensity. If the processes of transforming organic material are equated to the decomposition of hexatomic hydrocarbons, it may be assumed that this intensity of CO_2 liberation corresponds to processing 70 to 152 mg of glucose per square meter per hour. The organic matter in the soil, however, chiefly represents the remnants of animal and plant decay. An indirect indication of the presence of organic material is the percentage of total nitrogen in a soil specimen. Table 4 also gives these figures. Our data are close to those for total nitrogen determined by Feher (Ref. 6) for Sahara sands (from 0.02 to 0.05%). The quantity of organic material in the desert soils is negligible, but there is enough of it to make it possible for the microorganisms existing there to manifest themselves actively. /506

TABLE 5. STORAGE CONDITIONS OF STANDARD TEST TUBES WITH SOILS UNDER ANALYSIS

Site	Storage time of test tubes at temperature		
	30°	27°	4°
Darvaza	6 days	4 days	188 days
Repetek	9 days	4 days	179 days
Dushak	4 days	4 days	171 days

The next task was to ascertain which is more important for microorganisms -- organic material or moisture -- and what lower limit of moisture and organic material concentration is needed for starting mass reproduction

TABLE 6. NUMBER OF CELLS IN STANDARD TEST TUBES AT TERMINATION OF EXPERIMENT, MILLION PER GRAM

Sample sites and initial cell count, million/g	Hygroscopic coefficient %	Desert soil (0.3521% of total N)	Lava (0.1209% of total N)	Limonite (traces of N)	Garden soil (10.8581% of total N)
Darvaza, 63.5	0	239.2	64.3(?)	259.4	164.3
	1	96.3	-*	187.8	190.5
	5	127.1	227.6	268.2	272.8
	10	227.6	-	432.1	470.8
Repetek, 84.1	0	-	216.5	251.7	213.2
	1	145.9	-	199.6	115.8
	5	471.9	339.1	405.6	291.7
	10	806.2	605.5	571.4	-
Dushak, 187.8	0	493.5	531.3	-	174.8
	1	410.9	-	564.2	833.9
	5	661.3	522.1	721.6	-
	10	1996.5	925.6	855.1	435.0

* Dash indicates that no determination was made.

of the deserticolous bacteria. As already noted, standard test tubes with sterile soil having a definite percentage of organic matter and a given humidity had been prepared in advance for this experiment. Table 5 gives the storage periods of the standard test tubes from the moment that the experimental specimens of desert soil were introduced into them until the beginning of microbiological analysis.

The storage period leaves no doubt that during this period the cells capable of multiplication should have increased in number. At the end of the storage period an analysis was conducted both by the direct counting method and by inoculation in dishes of meat-peptone agar. The colonies cultured in the dishes were not distinguished by great variety, but pigmented forms were encountered more frequently than in analyses of soils -- particularly in samplings where mountain wilderness soil was taken as the standard. The colony number in the dishes was also limited, even in the case of garden soil rich in organic material. Therefore, only the results of direct cell counting were used for the findings given in Table 6.

The first column of the table gives the initial number of cells in the soils investigated which were inserted in the standard test tubes. Thus, the increase in cell count because of organic material and moisture in the standard test tubes is the difference between the first and the following columns /507

of the table. On the basis of the findings shown, it may be noted that the addition of organic matter in the form of natural humus of garden soil without the addition of water does not lead to a rise in the microorganism count. The addition of water in an amount of less than 5% of absolute dry soil weight does not affect the number of cells. The moisture limit required for a perceptible increase in cell count is around 5%.

It appears that water is a more important factor than the presence of organic matter for starting the division of microbe cells. The threshold moisture value which we discovered to be necessary for the start of microorganism multiplication is close to the figures quoted by Novogrudskiy (Refs. 2 and 3). The author notes that fungi are more drought resistant than are bacteria. He observed development of fungi at moistures of not less than 3.36%, but his findings show that the lower moisture limit needed for the reproduction of bacteria cells varies for soils with different humus contents. The greater the humus concentration, the more moisture is required for the start of bacteria multiplication. Our observations are also similar to this: a moisture rise in the test tubes with soils poor in organic matter had a greater effect on the multiplication of microbe cells than did the addition of water to the garden soil.

Conclusions

1. The upper layers of the Turkmenian desert where total nitrogen content is no more than 1.1% and the sand moisture fluctuates from 0.1 to 2.7% are populated by viable microorganisms.

2. Microbe cell viability is retained at a daily soil temperature of 60°C, diurnal temperature differentials of 33°C, and maximum insolation.

3. Moisture is the more important factor for microbe viability than is the presence of organic matter.

4. The moisture limit necessary for the start of bacteria cell multiplication is between 1 and 5%.

5. Colonies cultured in inoculations of the investigated specimens on meat-peptone agar differ only slightly and have weak pigmentation.

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